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The objectives of this study are to (1) analyze gene expression in a model of human ovarian carcinogenesis from a benign to a malignant phenotype in organotypic culture and (2) to confirm gene expression patterns by RNA analysis. Sufficient RNA for microarray analysis has been isolated and purified and we changed from cDNA arrays to oligonucleotide arrays. All RNAs from one cell line (96.9.18) have queried the Affymetrix human U133 A and B chips in triplicate. All RNAs from the second cell line (1.24.96) have queried the Affymetrix U133A plus chip in triplicate. Data mining is underway by the Bioinformatics Core at Wayne State University. Analysis of the queries of the U133 A and B chips indicate that 231 genes are differentially expressed between benign and malignant cells when data transformation is done by ANOVA at $p \leq 0.1$ with an expression level of >1.2-fold change. Quantitative real time polymerase chain reaction validated the expression levels of all genes tested. About 60% of the genes we have identified have previously been shown to be dysregulated in cancer and about 50% of those have been shown to be dysregulated in ovarian cancer thus validating our model.

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Introduction: Most epithelial cell cancers (cervix, colon, skin, prostate, breast, etc.) develop from precursor lesions resulting from an accumulation of mutations in growth regulatory genes. Such precursor lesions have not been identified for OVCA but it has been proposed that OVCAs arise by a multistep process through increasingly aggressive stages. We have shown that immortalized human ovarian surface epithelial (HOSE) cells undergo stepwise progression to the malignant phenotype in vitro. This process has been reproduced with a mouse ovarian cancer model we have developed. Our preliminary results with gene expression analysis of HOSE cells confirms our hypothesis that this phenotypic presentation reflects changes in the expression of genes from benign cells to malignantly transformed cells. The long-range goal of these studies is to identify aberrantly expressed genes in HOSE cells at various stages along the path to the malignant phenotype for the purpose of characterizing biochemical pathways whose expression is dysregulated.

Body: Our progress to date in analysis of gene expression of HOSE cells is provided in the Table below.

HOSE cell	Passage	RNA isolated	RNA labeled	Affymetrix array	Data analysis
96.9.18	Low	yes	yes	yes	yes
	Intermed.	yes	yes	yes	yes
	Late	yes	yes	yes	yes
1.24.96	Low	yes	yes	yes	pending
	Intermed.	yes	yes	yes	pending
	Late	yes	yes	yes	pending

Triplicate cultures of HOSE cells (96.9.18 and 1.24.96) from early, intermediate and late passages were cultivated on collagen rafts to confluency and then raised to the air medium interface and grown for three weeks. Extracted RNA was purified and labeled per the Affymetrix Eukaryotic Target Preparation guide and hybridized to either the Human Genome U133 (HG-U133A & B) Set (96.9.18) which contains almost 45,000 probe sets representing more than 39,000 transcripts derived from approximately 33,000 well substantiated human genes or the Human Genome U133 Plus 2.0 array that is identical to U133 A & B but contains an additional 6,500 transcripts in a single chip. Chips were scanned with an Affymetrix GeneChip 3000 scanner. Data for 96.9.18 was normalized using dChip and ANOVA (GeneSpring). GeneSpring software was used to identify genes with >1.2 fold change and a p value ≤ 0.1. Validation of expression of selected genes was done by quantitative real-time polymerase chain reaction (QRT-PCR) using Sybr Green detection of product. There were 101 genes differentially expressed when early and intermediate passage cells were compared and 231 gene differentially expressed when intermediate and late passage cells were compared. The 101 differentially expressed genes from the early/intermediate comparison formed a subset of those found in the intermediate/late comparison. Some of the genes that were identified as over expressed in our model and found overexpressed in ovarian cancer include the cytokines IL8 and IL6, APOE, CLDN1, CDA and PP1R15A. Underexpressed genes include the keratins KRT6 and KRT18, GAL1, DCN and TAGLN.

Validation by QRT-PCR showed, on average, a 40-fold difference in expression levels between PCR and Affymetrix. For example, IL8 showed a 80-fold increase in expression between early and late

passage cells by Affymetrix but a 3800-fold difference by QRT-PCR. Likewise, DCN showed a 25-fold decrease between early and late passage cells by Affymetrix but a 352-fold decrease by QRT-PCR. Thus, Affymetrix can be consider semi-quantitative and it is likely that genes that show only a minor change by gene chip analysis may have a significant change when assayed by QRT-PCR

Key Research Accomplishments:

- Have demonstrated that our RNA isolation procedures are compatible with labeling protocol used for Affymetrix oligonucleotide arrays.
- Have successfully queried the U133A and B chips with 96.9.18 RNA and the U133 Plus 2.0 chip with 1.24.96 RNA for early, intermediate and late passage cells.
- Analysis of the 96.9.18 data indicates 231 genes differentially expressed between early and late passage cells many of which have previously been shown to be associated with either ovarian cancer or cancer at other sites.
- Dysregulated genes could be verified by QRT-PCR.

Reportable Outcomes: Abstract: Gregoire L, Thota A, Lancaster WD. Changes in gene expression during progression to te malignant phenotype in a human ovarian surface epithelial cell model. American Association for Cancer Research, Advances in Cancer Research, Waikoloa, Hawaii, January 2004.

Conclusions: We have requested and received a 12 month no cost extension because of difficulties with tissue culture that were beyond our control that made completion of the project in the 36 month time period impossible. For the third 12 months of the award, we have completed all queries of the Affymetrix oligonucleotide arrays with two cell lines. Preliminary analysis of the results of these hybridizations indicated a number of genes that are differentially expressed in early, intermediate and late passage cells and that these differences can be validated by QRT-PCR. For the next 12 months we will complete the analysis of 1.24.96 cell data and will have submitted abstracts and mauscripts. The results to data indicate that new avenues of investigation can be initiated to dissect development of ovarian cancer as well as development of therapeutics and early biomarkers of early ovarian cancer. The lack of description of a "premalignant" ovarian surface epithelial cell phenotype in situ has hindered progress in early diagnosis of epithelial ovarian cancer. The cell culture system we have developed mimics a premalignant condition in that there is local invasion of matrix by early passage cells. These cultures will be of value in identifying early changes in biochemical pathways that become dysregulated early during malignant progression.

References: None

Appendices: One abstract

6th Joint Conference of the American Association for Cancer Research and the Japanese Cancer Association

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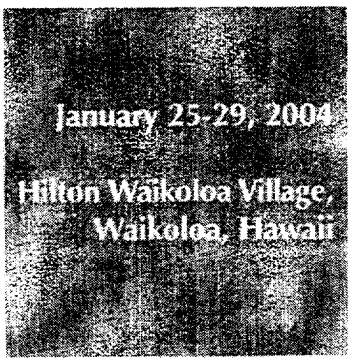

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proximal to the multidrug resistance gene mdr1a, is an initial event that triggers mdr1a amplification through the breakage-fusion-bridge (BFB) mechanism. To investigate the molecular mechanism underlying the initial events of mdr1a amplification, we have cloned portion of 1q31 fragile site DNA. Strikingly, we found that this fragile site contains a novel gene, designated as fragile site associated (FSA) gene: (i) FSA encodes an unusually large mRNA of ~16 kb. (ii) Human FSA mRNA contains two nonoverlapping, evolutionarily conserved open reading frames (orf1 and orf2). (iii) FSA is preferentially expressed in postmitotic spermatocytes. (iv) While the function of orf1-encoded protein is unknown, orf2 shares striking amino acid similarity to that of Caenorhabditi elegans lipid depleted-3 (lpd-3) gene. These results suggest a complex genomic organization of 1q31 locus regulating mdr1 amplification and fat metabolism. Keywords: fragile site, DNA amplification, mdr, biocistronic, lipid depleted-3

C40

Proteome Analysis of Esophageal Cancer with Two-dimensional Differential Gel Electrophoresis using Agarose Gel

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One of most important subjects for control over cancer is establishment of early detection method using clinical sample such as serum or urine. According to development in mass spectrometry technology, numerous proteome studies recently have been performed to identify disease-related changes in protein expression. The aim of this study is to find some novel biomarkers, which are useful for early detection of esophageal cancer using proteome technique. Two-dimensional differential gel electrophoresis (2D-DIGE) using agarose gel for IEF was performed to analyze protein expression of primary esophageal cancer tissues. 2D-DIGE is a novel technique to separate two pools of proteins labeled with different fluorescent dyes and identify differentially expressed proteins. Two-dimensional gel electrophoresis (2DE) using agarose gel for IEF has advantages of higher loading capacity than IPGs gel. Additionally, it is also able to resolve basic proteins and high molecular mass proteins larger than 150kDa, which are difficult to resolve with IPGs. Using these techniques, protein extracts from tumor and corresponding non-tumor tissues, which were surgically resected from 12 cases of primary esophageal cancer, were separated. Written informed consent was obtained from each patient prior to surgery. The gel images were scanned with a fluorescent image scanner (Typhoon 9400™, Amersham Biosciences, Inc.) and were analyzed with DeCyder™ (Amersham Biosciences, Inc.). About 400 protein spots were differentially expressed between matched tumor and non-tumor tissues. These proteins were analyzed using mass spectrometry and several novel proteins were identified. These findings would be helpful not only to develop new biomarkers or therapeutic drugs but also to understand the underlying mechanism of carcinogenesis.

C41

Changes in Gene Expression during Progression to the Malignant Phenotype in a Human Ovarian Surface Epithelial Cell Model

Lucie Grégoire, Aditya Thota, Wayne Lancaster, Wayne State Univ. Medical School, Detroit, MI.

We have developed an in vitro human epithelial ovarian cancer model by growth of HPV-16 E6/E7 immortalized human ovarian surface epithelial (HOSE) cells in an organotypic (collagen raft) culture system (Gregoire et al. 2001. Clin. Cancer Res. 7:4280). At early passage, cells form a monolayer on collagen rafts. At intermediate passage there is focal stratification and single cell invasion of the collagen. At late passage, cells form colonies in soft agar and form mounds of invading cells on collagen. These later stage cells form tumors in immunodeficient mice. To understand what gene expression changes occur during this phenotypic progression we have queried Affymetrix U133B chips with low and intermediate passage HOSE

cell RNA. Three independent cultures of the same passage were grown in parallel on collagen rafts and the RNA isolated and labeled in parallel. Parameters of the chip data that are used to evaluate the hybridization were all within limits indicating no degradation of RNA, sufficient level of label and low background. Comparison of a baseline data set with other 5 data sets showed a correlation of 0.8 indicating that only a small percentage of genes would be differentially expressed. The raw data was subjected to two different statistical analyses: t-test with an adjusted p value of 0.05 and a confidence analysis. The t-test showed that between the low and intermediate passage cells 180 genes were differentially expressed. The confidence analysis showed 210 genes were differentially expressed. The intersection of these two data sets showed that 20 genes were common to both methods of data manipulation. Of the 20 genes, 9 are known and 11 are either expressed sequence tags or hypothetical proteins. Of the 9 known genes, most are involved in cell growth, proliferation, differentiation, longevity, and transformation. This preliminary analysis is being expanded to include U133A chips and comparison of all three phases of phenotypic change.

C42
Probabilistic Analysis of Array-CGH Profiles Identifies Genomic Alterations Specific to Stage and MYCN-Amplification

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Recurrent non-random genomic alterations are the hallmarks of cancer and the characterization of these imbalances is critical to our understanding of tumorigenesis and cancer progression. Here we performed Array-comparative genomic hybridization (A-CGH) on cDNA microarrays containing 42,000 elements in neuroblastoma. We applied a novel probabilistic algorithm, topological statistics, to increase the sensitivity of cDNA A-CGH for detecting single-copy alterations. Our method not only overcomes the shortcoming of relative low sensitivity of cDNA A-CGH but also enables a direct visualization of the statistical confidence for the observed alterations. Furthermore using a probabilistic approach to estimate the frequency of genomic imbalances in tumors of different stage and *MYCN* amplification status, we identified unique patterns of genomic imbalance in all three categories. Finally, from a list of all possible hierarchical models of tumor progression, we identified a model that best fits our data to generate a hypothesis on the evolution of neuroblastomas.

C43
ONCOMINE: A Cancer Microarray Database for Therapeutic Target and Biomarker Discovery

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DNA microarray technology has led to an explosion of oncogenomic analyses, generating a wealth of data and uncovering the complex gene expression patterns of cancer. Unfortunately, due to the lack of a unifying bioinformatics resource, the majority of this data sits stagnant and disjointed following publication, massively under-utilized by the cancer research community. Here, we present *ONCOMINE*(http://www.oncomine.org), a publicly available cancer microarray database and web-based data-mining platform aimed at unifying data and analysis, and facilitating therapeutic target and biomarker discovery from genome-wide expression analyses. To date, *ONCOMINE* contains 65 published cancer gene expression datasets, comprising nearly 50 million gene expression measurements form more than 4,700 microarray experiments. From this data, more than 100 differential expression analyses have been conducted, identifying gene expression signatures for most types of cancer as well as for a variety of cancer subtypes, including many defined by clinical, pathological and genetic criteria. While some of the signatures available in *ONCOMINE* are analogous to those described in the original publications, many represent alternative